

Standard operation procedure: Preparing for rederivation of rats by caesarian section

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PREPARING FOR REDERIVATION OF RATS BY CAESAREAN SECTION

1.0 PURPOSE

- 1.1 Rederivation by caesarean section is performed in order to purify one or more unique lines shown to contain undesirable pathogens.
- 1.2 This procedure is to ensure that all stages carried out prior to the caesarean section are well planned.

2.0 DIVISION OF RESPONSIBILITY

- 2.1 The Head of Department is responsible for the procedure. The Head of Department coordinates the procedure or delegates this to another person.
- 2.2 In collaboration with the other involved parties, the person responsible for coordinating the procedure must draw up a written plan for preparing and performing the caesarean in good time before it is carried out.
- 2.3 All those involved must fully understand their role in the procedure and make sure that the various stages are carried out according to plan.

3.0 PROCEDURE

- 3.1 A rederivation plan must be initiated immediately when undesirable pathogens are discovered and a step-by-step elimination is required. Strict hygiene measure must be applied in order to minimise the risk of contaminating the surrounding environment. A lab in the immediate vicinity of the room in which the infected animals are housed is designated for use by the affected animals.
- 3.2 The Head of Department contacts the users and informs them that rederivation is necessary in order to re-establish clean lines.
- 3.3 The Head of Department, the person with special screening responsibility (PMSK) and other relevant personnel draw up a plan for the procedure that is distributed to the other members of staff at KPM (Section for Comparative Medicine). A number of named persons are selected to carry out the various tasks: moving clean animals, cleaning rooms, ordering equipment and animals, breeding plan, plug check, weighing female animals, injecting Depo-Provera (medroxyprogesterone to stop birth), caesarean section etc.
- 3.4 The applicable SOPs must be read and understood of all those involved. A meeting must be convened to discuss all details and questions pertaining to the procedure.



- 3.5 A suitable room for housing the rederived animals and the purchased, clean animals belonging to the relevant users is emptied and disinfected. The foster mothers (purchased animals) are housed in this room.
- 3.6 The date for carrying out the caesarean section on donor animals (i.e. the line to be rederived) should be fixed according to the strain and the operation should be carried out on the day after the foster mother has given birth. Rat strains often have different gestation periods. Knowledge of the strain in question should be used to make sure that the donor animals give birth ca. the day after the foster mother gives birth.
- 3.7 Five named people must be selected to perform the following tasks: person 1 despatches donor females from the dirty housing room; person 2 moves the donor female from the dirty housing room and the dirty lab + moves rederived young to the clean housing room; person 3 and person 4 perform the caesarean section; person 5 receives the pups in the clean housing room. See SOP 15-03 "Rederivation of rats by means of caesarean section» for a detailed description of how the caesarean section is carried out.
- 3.8 Make sure the necessary equipment is in place. Any missing equipment (see the list below) must be acquired:
- Sterile Q-tips
 - Physiological saline
 - Microscope slides
 - Crystal violet solution, Sigma-Aldrich
 - Microscope (200x)
 - CO₂ apparatus
 - Virkon
 - Bottom of an empty GM500-cage (for disinfecting the womb)
 - Bottom of an empty 900-cage (for disinfecting the foster mother)
 - Autoclaved paper for wrapping surgical instruments
 - Instrument set 1: 2 pairs of scissors (one small and one large/two small ones) and 2 forceps (one surgical and one anatomical)
 - Instrument set 2: one small pair of scissors and 2 forceps (one surgical and one anatomical)
 - Sterile aprons/lab coats
 - Sterile gloves
 - Heat pad
 - Sterile matting/autoclaved paper for covering the work top and heat pad
 - Sterile water
 - Glass bead sterilizer with glass beads
 - Dopram (stimulates respiration) in pipettes
 - 500-cage for transporting pups
 - Depo-Provera

- 3.9 The breeding of animals for training purposes is arranged: plug check, caesarean section and transfer to the foster mother. The training must be carried out in good time before the rederivation is to take place.
- 3.10 The PMSK orders a sufficient number of 14-week old Sprague Dawley females (with or without birth experience) with SPF/SOPF microbiological status and 14-week old Sprague Dawley males with SPF/SOPF microbiological status. These are housed in a clean room. The necessary time for acclimatisation after arrival is minimum 3 days. Ear tag the females so that that one can be distinguished from the other.
- 3.11 A plan is drawn up for the breeding of foster mothers (purchased animals) and donor animals (the line to be rederivated).
- 3.12 Book the lab for performing the caesarean section in good time.

Breeding and following up the foster mothers

- 3.13 The breeding plan must take account of the fact that not all of the animals will become pregnant. The recommended number of females is four per line.
- 3.14 Litter from the male's cage must be introduced to the females 7 days before mating is to take place. House the male alone so that he can mark out a territory.
- 3.15 Draw up a plan for trio breeding with foster mothers. Weigh the females and register their weight on the cage card and in SL. Select females that are in oestrus (in heat). Oestrus can be monitored by checking the appearance of the vaginal opening (see figure 1), cell morphology in the vagina ([Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification \(nih.gov\)](#)) or the animals' mating receptivity.
- 3.16 A plug check of the foster mothers should be carried out the day after mating and up to two days after breeding is set up. If one of the females is vaginal-plug positive (vp+), this animal is taken out and the cage is labelled with the date of the vp+ and the expected birth date (ca. 21-23 days after the vp+ date). SL must also be updated with this data. If both the females are vp+, the male is taken out. Put the females in the same cage. Breeding ends on Wednesday, even if one or both females are vp negative (vp-).
- 3.17 Weigh the females on day 7 and 10 after mating in order to confirm/rule out pregnancy. A new breeding session with plug check is planned if necessary.
- 3.18 The foster mothers are placed in individual, clean cages on the Friday before the expected birth.
- 3.19 The foster mothers will hopefully give birth on day 19 or on one of the days immediately following. Daily monitoring must be carried out to check for births and that everything is in order. Register the birth(s) in SL and inform all those involved.

Breeding and following up donor animals

- 3.20 Make sure that the females are ear tagged so that they can be distinguished from one another. The breeding plan must take account of the fact that not all of the animals will become pregnant.
- 3.21 The donor animals are housed in the dirty housing room. Litter from the male's cage must be introduced to the females on Wednesday, 7 days before mating is to take place. House the male alone so that he can mark out a territory.

- 3.22 Trio breeding of donor animals is set up 2 days after the planned breeding date for the foster mothers. Weigh the females and register their weight on the cage card and in SL. Place the females in the male's cage. Select females that are in oestrus (in heat). Oestrus can be monitored by checking the appearance of the vaginal opening (see figure 1), cell morphology in the vagina ([Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification \(nih.gov\)](#)) or the animals' mating receptivity.
- 3.23 Take out the females that are vp+. Label the cage card with the vp+ date and the expected birth date (ca. 21-23 days after vp+) and also update SL. Put the females in the same cage. Breeding ends on Friday, even if both the females are vp negative (vp-). New breeding with plug check should be set up if necessary.
- 3.24 Weigh the females on day 7 and 10 after mating in order to confirm/rule out pregnancy. The date for performing the caesarean section is confirmed. Another meeting is held with all those involved. Confirm the booking of the lab and change the booking date if necessary.
- 3.25 The plan must be set up in such a way that the expected birth date of the donor female falls on the day after the foster mother has given birth. On day 3 before the expected birth date, the donor female should be injected with Depo-Provera 0,01 ml/300g s.c. to postpone the birth. House the donor females in individual cages.
- 3.26 If a donor female gives birth unexpectedly, a new rederivation plan must be drawn up.

4.0 HEALTH, SAFETY AND ENVIRONMENT (HSE)

- 4.1 Everyone must have undergone sufficient training to ensure the correct use of clothing and protective equipment.
- 4.2 Everyone handling animals must have undergone sufficient training and practice to ensure that animals are handled correctly
- 4.3 The work must be carried out on a ventilated worktop or LAF-bench in order to minimise exposure to allergens and agents and to limit the spread of such to the surroundings.

5.0 EQUIPMENT AND MAINTENANCE

- 5.1 900-cages
- 5.2 Large water bottles with a ball in the cap
- 5.3 Enrichment
- 5.4 Microscope slides
- 5.5 Crystal violet solution, Sigma-Aldrich
- 5.6 Microscope (200x)
- 5.7 Depo-Provera
- 5.8 SL

6.0 HISTORY AND EDITING

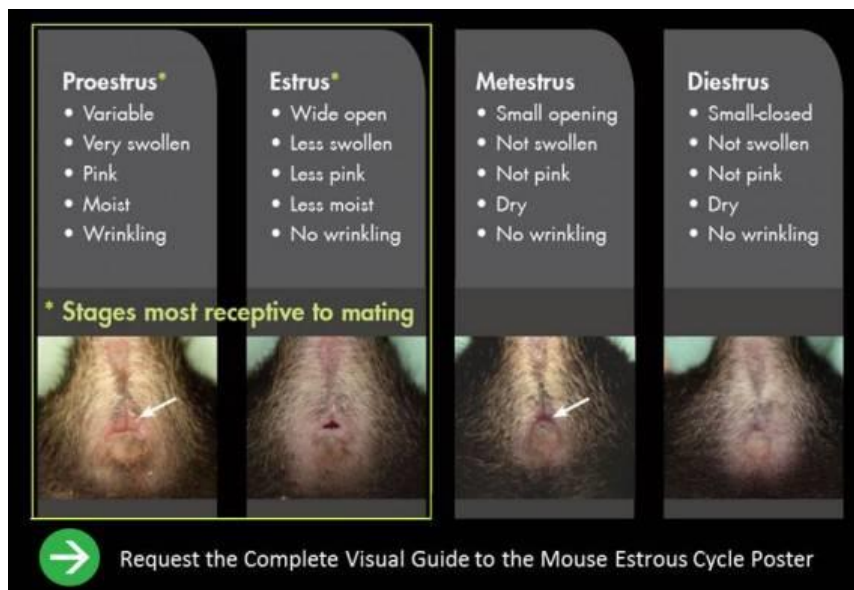
- 6.1 Sections of this SOP are based on the SOP "Keisersnitt ansvar oppstalling passasje", written by Mikael Westberg 10.09.2014

6.2 20.05.2021, Frøydis Kilmer: Major amendments to the original SOP (“Keisersnitt ansvar oppstalling passasje”) incorporated into “Preparing for rederivation of rats by caesarian section”

7.0 REFERENCES

- 7.1 Regulations governing the use of animals in scientific experiments, last amended FOR-2010-08-06-1147
- 7.2 APPENDIX A to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Guidelines for the accommodation and care of animals
- 7.3 DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes
- 7.4 Phased elimination plan for Streptococcus in DU-045
- 7.5 Rederivation of rats by caesarean section
- 7.6 6 steps for setting up timed pregnant mice (jax.org)

Figure 1: Oestrus cycle in mice



<https://www.jax.org/>